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Effects of genotype x bradyrhizobium inoculation or x fertilizer n interactions on genetic gains while selecting in soybean

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Abstract

Introduction of soybean *(Glycine max* (L.) Merr.) to environments where it was not previously produced sometimes occurs without successful introduction of the symbiont, *Bradyrhizobium japonicum* (Kirchner) Buchanan. Plant breeders working in such environments need to know whether relative performance of soybean genotypes is affected by the presence or absence of *B. japonicum*. The primary objective of these studies was to assess genotype x inoculation interactions and expected genetic gains for a soybean population grown with and without inoculation on a soil free of *B. japonicum*. A second objective was to determine whether the response of soybean genotypes to N depend on the source of N (fertilizer or symbiotic fixation). A set of 52 random lines from a broad-based reference population was evaluated for 2 years with and without *B. japonicum* inoculation in a split-plot design. Inoculation increased protein content and lodging and decreased oil content. Genotype x inoculation interaction was significant only for protein and oil content in one year. Expected genetic gains were similar with and without inoculation. A second set of 12 genotypes was evaluated for 2 years in a similar design but with N fertilizer applied as a third N treatment. There were no significant differences among N treatments for any trait. Genotype x inoculation interaction occurred for yield, but not for other traits. Genotype x inoculation interaction for seed yield and composition may occur in some environments but is not a major constraint to genetic gain.

Key words: *Bradyrhizobium japonicum*, Fertilizers, *Glycine max*, Genotype x : Inoculation Interactions, Nitrogen fixation

Introduction

A soybean breeder charged with the development of cultivars is required to consider adaptation of soybean genotypes to two types of environments, characterized by presence and absence of *B. japonicum*. Research conducted in both temperate and tropical environments indicated that inoculated soybean derives most of its N from symbiotic fixation (Welch *et al.*, 1973; Chesney, 1973; Specht *et al.*, 1999). Unfortunately, co-introduction of the symbiont *B. japonicum* has been delayed in some cases, because of difficulty in production and distribution of the rhozobium inoculant. For example, soybean was introduced into Uganda between 1908 and 1913, but many production fields lack *B. japonicum*. Although a national program is working to establish ways to deliver inoculum to Ugandan growers inexpensively, soybean may continue to be grown for many years on uninoculated soils. Further, since little N fertilizer is used in Uganda, absence of *B. japonium* is expected to constitute a stress for the soybean crop. Yield increases of approximately 1 t ha^{-1} due to inoculation of seed have been reported in Uganda (G. Gumisiriza and M. Mbalule 1989, unpublished data). Since soybean in Uganda is utilized primarily as a source of protein, it is important to assess the effect of inoculation on protein content of the seed.

Differences among soybean genotypes inability to nodulate with either all strains or specific strains of *B. japonicum* have been reported (Palmer and Killen, 1987; Cregan *et al.*, 1989). There is limited information, however, on effects of genotype x inoculation interactions on yield, seed composition, or other agronomic traits including plant height, lodging, dates to flowering and to maturity *inter alia*. It is unclear, for example, whether breeders should select in the presence or absence of *B. japonicum*. If interactions are important, it is possible that cultivars selected in one type of environment may not be ad ıpted to the other type. This situation might call for separate breeding programs for the two types of environments.

In the absence of *B. japonicum*, producers may attempt to supply the soybean with N through fertilizer applications. Breeders also need to know whether soybean genotype interacts with sources of N to influence economic traits.

The primary objective of this study was to assess genotype x inoculation interactions and expected genetic gains for protein content and other traits of soybean genotypes grown with and without inoculation on a soil free of *B. japonicum*. A second objective was to determine whether response of soybean genotypes to N depended on whether N was derived from fertilizer or symbiotic fixation.

Materials and methods

Two sets of genetic materials were used. A set of random lines from a broad-based population was evaluated with and without *B. japonicum* (random-line experiment), and a set of 12 cultivars and advanced breeding lines chosen to represent a wide range of seed composition was evaluated in combination with three N treatments (cultivar experiment).

The experiments were conducted during 1991 and 1992 on adjacent fields at Jackson, Ohio, U.S.A on an Omulga silt loam soil (fine-silty, mixed mesic Typic Fragiudalfs). These fields had been under grass and alfalfa (*Medicago sativa* L.) for several years and had not previously been cropped with soybean. Uninoculated soybean planted in a sample of this soil in spring 1991 did not produce nodules. In both years, P and K were applied at rates derived from standard recommendations based on soil tests. In addition, because soil tests conducted in spring 1991 revealed little amounts of nitrate, Urea was applied at a rate of 22.5 kg N/ ha in 1991. This fertilizer was applied to the entire field prior to ploughing.

Random-Line experiment

Lines for the random line experiment were derived from the population AP10C2, which in turn was derived from the germplasm population AP10 (Fehr and Rodriguez de Cianzio, 1981). The parents of AP10 were 40 plant introduction strains, originating from eight different countries and selected for high yield in Iowa USA. Beginning in 1980, AP10 was subjected to two cycles of recurrent selection for yield, maturity, and lodging resistance in Ohio USA, resulting in population AP10C2. Recurrent selection practices consisted of evaluation of $S_{2:}$ or $S_{1:}$ derived lines in hill plots, followed by a second year's evaluation in two-row plots at two locations. This was further followed by a single intermating generation. A random set of 60 $S_{4:}$ derived lines was obtained from AP10C2 for this study.

In 1991, the 60 random lines and four control genotypes, 'Century 84', 'Williams 82', HS89-2958, and HS84-6247, were arranged in a split-plot design with two replications. Inoculation treatments (presence and absence) were assigned to whole plots in two blocks. Genotypes were assigned to sub-plots. Each sub-plot was a single row, 0.9-m long, with a spacing of 1 m between sub-plots. Planting rate was approximately 20 seeds m^{-1}. The plots were planted by hand on 31 May 1991. In the inoculated treatment, approximately 2 ml of a liquid suspension was applied to each seed in the furrow prior to covering it. The suspension contained approximately 10^8 cells/ml of *B. japonicum*, strains 61A76 and USDA 123 in a 1:1 ratio of cells. The bradyrhizobial cells had been cultured in a yeast-extract-mannitol-gluconate nutrition medium at pH 6.5 to 7.0.

At the R3 growth stage (Fehr and Caviness, 1979), one plant was uprooted from each plot and scored for extent of nodulation on a 1 to 5 scale, where 1 indicated no nodules and 5 indicated abundant nodulation. Twelve nodules were removed from each nodulated plant to determine which strain was present. Nodules were sterilized in a calcium hypochlorite solution, rinsed five times in distilled water, and individually crushed with a sterilized glass rod in sterilized water. Bacterial extracts from each nodule were dropped on yeast-extract-mannitol-gluconate media solidified in agar in three petri dishes, one containing spectinomycin, one streptomycin, and one a control. The growth of the rhizobium was assessed after incubation for three days at 29°C. Strain 61A76 was resistant to streptomycin and USDA 123 was resistant to spectinomycin (Bhuvaneswari *et al.*, 1983); growth or failure to grow on these antibiotics therefore indicated the strain(s) occupying each nodule. Chi-square tests were used to assess genotypic differences in occupancy of nodules by the rhizobium strains.

The date of maturity, plant height, and lodging score for each plot were recorded. Date of maturity was the date on which 95% of the pods attained their mature colour. Plant height was measured after maturity. Lodging was rated on a scale of 1 (erect) to 5 (prostrate). A sample of seed from each plot was analyzed for protein and oil content at the Ohio State University Grain Quality Laboratory, Wooster, Ohio, USA, using near infrared transmittance. Protein and oil content were expressed on a dry-matter basis. No data on yield were taken in the 1991 season. This was due to the small plot sizes used with limited amount of seeds at hand, which would not offer accurate comparisons for seed yields among the treatments.

The experiment was repeated in an adjacent field in 1992. Eight of the AP10C2 entries were eliminated due to insufficient seed, leaving 52 AP10C2 entries and the 4 controls. The treatments were again arranged in a split-plot design with two replications, with inoculation as the main-plot factor. Each subplot consisted of six rows, 2.7 m long, spaced 38 cm apart. Planting rate was approximately 20 seeds m^{-1}. The trial was planted mechanically on 28 May 1992, with inoculation delivered by a vermiculite carrier (Graham-Weiss *et al.*, 1987). Approximately 12 g of the inoculum were used per kg of seed. The inoculum contained approximately 10^9 *B. japonicum* cells g^{-1}, from strains 61A76 and USDA 123 in approximately 1:1 (cell:cell) ratio.

In 1992, the same data was collected as in 1991, in addition to seed yield and 100-seed weight. Each subplot was trimmed to a length of 1.5 m after physiological maturity and the inner four rows were harvested with a plot combiner to obtain seed yield. Subsequently, a split-plot analysis of variance was performed for each year and across years (McIntosh, 1983). Years and genotypes (excluding control varieties) were considered random factors.

In addition, the data for each inoculation treatment was analyzed separately, excluding control genotypes, to obtain estimates of variance and covariance components and correlations. Expected genetic gain in trait y on selection for trait x was determined as $D_{yx} = iCov_G(x,y)(s_{GX}^2+s_{GYX}^2/m + s_{ex}^2/rm)^{-½}$, where $i = 1.755$ (the standardized selection differential for 10% intensity), $Cov_G(x,y)$ = the genotype covariance between x and y, r = the number of replications per environment, m = the number of test environments, and s_{GX}^2, s_{GYX}^2, and s_{ex}^2 refer, respectively, to genotypic, genotype x environment, and error variance components for x. Two types of selection were assumed: (1) selection

based on a family mean with r=2 and m=2, and (2) selection based on unreplicated plots with r=1 and m=1. When x was yield, the formula was modified to $D_{yx} = iCov_G(x,y)(s_{G_x}^2 + s_{gyx}^2/r)^{-1/2}$ and family-mean selection was assumed to have r=2, while the value r=1 was used for single-plot selection. Since yield was measured only in 1992, variance components and expected genetic gains involving yield are subject to bias due to failure to measure genotype x environment interaction.

Standard errors of variance components and expected genetic gains were obtained as indicated by Mode and Robinson (1959). Two variance components V_1 and V_2 were considered to differ significantly if $|V_1 - V_2| > 2[(SE_1)^2 + (SE_2)^2]^{1/2}$, where SE_1 refers to the standard error of V_1. A similar procedure was used to compare expected genetic gains. The effect of inoculation treatments on phenotypic correlations was tested using the normal transformation (Steel and Torrie, 1980).

Cultivar experiment.

The experiment consisted of varieties and breeding lines with relatively high ('Vinton 81', 'Century 84', HS89-2958, and HS89-2987), intermediate ('Resnik', 'Edison', Williams 82, and 'Keller') and low ('Conrad', 'Kenwood', 'Sandusky', and HS84-6247) seed protein content. Three N treatments were employed in factorial combination with the 12 genotypes. The first treatment, inoculation with *B.japonicum*, was applied in the same way as in the random line experiment each year i.e by application of a liquid suspension in 1991 or a vermiculite carrier of the rhizobium inoculant in 1992. The second treatment consisted of a side-dressing with urea at a rate of 284 kg N ha^{-1}, applied at the V2 growth stage (Fehr and Caviness, 1979). The third treatment was an uninoculated, unfertilized control.

A split-plot design with the N treatments randomly assigned in each of the two blocks was used. In 1991, each subplot consisted of three rows 3.6 m long, spaced 1 m apart. These plots were end-trimmed to a length of 2.1 m, and the inner row was harvested to determine seed yield. In 1992, each subplot had 6 rows 2.7 m long, spaced 38 cm apart. After physiological maturity, the subplots were trimmed to 1.5 m in length and the inner four rows were harvested to determine seed yield. Planting dates were 31 May 1991 and 28 May 1992. In addition to seed yield, days to maturity, lodging, weight of 100 seeds, and seed protein and oil content for each plot were determined.

Combined analysis of the cultivar experiment was carried out with genotypes and N treatments considered as fixed effects and years as random effects (McIntosh, 1983). Interplot error mean squares for seed yield were calculated for each of the three N treatments and examined for homogeneity using Bartlett's test (Steel and Torrie, 1980).

Results and discussion

Random-line experiment

Seedlings from all inoculated plots produced nodules, unlike in uninoculated plots. In both years, there were no differences among genotypes for extent of nodulation. In 1991, 955 nodules on AP10 genotypes were occupied by strain 61A76, and 494 nodules were occupied by USDA strain 123. In 1992, there were 779 nodules occupied by 61A76 and 631 by USDA 123. Chi-square tests revealed small but significant (P=0.05) genotypic differences in percentage of occupancy by the two strains, and all soybean genotypes were nodulated to some extent by each strain.

Combined across years, the effect of inoculation on seed protein and oil content was significant (P=0.05). For the inoculated treatment, the mean content of protein was 411 g kg^{-1} and that of oil was 206 g kg^{-1}. The means for the uninoculated treatment were 378 g kg^{-1} for protein and 219 g kg^{-1} for oil. Inoculation also significantly (P=0.05) increased lodging score from 2.5 to 3.2. The main effects of inoculation on maturity, plant height, and yield (1992 only) were not significant.

For protein and oil content, genotype x inoculation interaction was significant (P=0.05) in 1991 but not in 1992 or in the combined analysis (Table 1). For other traits, there was no significant genotype x inoculation interaction in either individual year or in the combined analysis.

Error variances for the traits of protein content, oilcontent and days to maturity were greater in uninoculated plots than in inoculated plots (Table 2). Error variances for protein and oil content were 6 to 8 times greater in the absence of *B. japonicum* than under inoculation. For maturity, there was a twofold difference in error variance.Genotype x year interactions were significant (P=0.05) only for height in the uninoculated treatment and for maturity, protein, and oil in the inoculated treatment. There was no trait, however, where the genotype x year variance component in the inoculated treatment differed significantly from its counterpart in the uninoculated treatment (Table 2).

In the case of seed yield, the F-test indicated significant (P=0.05) genotypic differences in the inoculated treatment but not in the uninoculated treatment. Genotypic differences were significant (P=0.05) for all other traits in both inoculation treatments, however, genotypic variance components did not differ between inoculation treatments (Table 2).

Table 1: Partial Analysis of variance for protein and oil content of 52 random soybean lines produced with and without *B. japonicum* inoculation.

		Mean Square	
Source	df	Protein	Oil
Inoculation	1	112 301**	16 801**
Inoculation x Year	2	2 457	867
Error a	2	8 200	1145
Genotype	51	1 559**	686**
Genotype x Year	51	354	79
Genotype x Inoculation	51	477	90
Genotype x Inoculation x Year	51	364	82
Error b	204	374	72

** Significant at the 0.01 probability level

Expected genetic gains for direct selection were not significantly different between inoculated and uninoculated treatments for any trait (Table 2). Yield gains in the uninoculated treatment were nearly zero, however, because of the small estimated genetic variance for yield. In 1992, inoculation significantly (P=0.05) decreased the phenotypic correlations between yield and protein content from 0.55 to 0.17 (Table 3). The correlation between yield and oil content underwent a corresponding change from –0.29 to 0.15. Inoculation may change the relative contributions of the physiological mechanisms underlying the relationship among yield, protein and oil content. It is possible that, under conditions of N stress induced by the absence of the symbiont, soybean genotypes possessing a mechanism for more efficient uptake of N from the soil are likely to rank high for both yield and protein content. Phenotypic correlations were not significantly different between inoculation treatments for other traits (Table 3). Correlations between protein and oil content were not as strongly negative in our study as in most previously reported experiments with soybean (Johnson and Bernard, 1963).

The effectiveness of selection for protein content with and without inoculation was evaluated by calculating expected genetic gains with both inoculated and uninoculated environments as targets. When selection was practiced in an inoculated environment, expected gain in an uninoculated environment was 21 g kg^{-1}, similar to the value of 21 kg^{-1} for direct selection in an uninoculated environment given in Table 2. When selection was practiced in an uninoculated environment, expected gain in an inoculated environment was 13 g kg^{-1}, similar to the value of 15 g kg^{-1} for direct selection given in Table 2. Therefore, irrespective of the target environment, a breeder can select for protein equally effectively in either type of selection environment (inoculated or un-inoculated environment).

Cultivar Experiment

As in the random-line experiment, there were no differences among genotypes in inoculated plots for extent of inoculation. There were, however, small differences (P=0.05) among genotypes in 1991 with respect to the

Table 2: Components of variance and expected genetic gains for AP10C2 Soybean genotypes evaluated for two years with and without *B. japonicum* inoculation.

				Expected Genetic Gain	
			Variance Component	Family	Single
B. japonicum	σ_e^2+	σ_{GY}^2+	σ_G^2	mean+	plot
			Protein (g kg^{-1})		
Absent	666 (92) '	-40 (73)	231 (79)	21 (5)	14 (2)
Present	87 (12)	23 (14)	98 (26)	15 (5)	12 (1)
			Oil (g kg^{-1})		
Absent	124 (17)	3 (15)	81 (23)	13 (3)	10 (1)
Present	20 (3)	5 (3)	73 (16)	14 (2)	13 (1)
			Yield (t ha^{-1})		
Absent	0.54 (0.10)	----------	0.006 (0.07)	0.02 (0.25)	0.01 (0.19)
Present	0.36 (0.07)	----------	0.111 (0.07)	0.36 (0.19)	0.28 (0.16)
			Maturity (d)		
Absent	15.6 (2.2)	0.5 (1.9)	71.7 (14.8)	14.5 (1.6)	13.4 (0.8)
Present	6.3 (0.9)	2.9 (1.3)	69.1 (14.0)	13.7 (0.8)	13.7 (0.8)
			Height (cm)		
Absent	180 (25)	43 (29)	233 (60)	24 (4)	19 (2)
Present	201 (28)	-13 (22)	225 (53)	24 (3)	19 (2)
			Lodging (score#)		
Absent	0.72 (0.10)	0.15 (0.11)	0.24 (0.11)	-0.6 (0.2)	-0.4 (0.1)
Present	0.74 (0.10)	0.05 (0.10)	0.56 (0.16)	-1.1 (0.2)	-0.8 (0.1)

=e = error, GY=genotype x year, G = genotype
Iselection intensity .10%; family mean selection based on two replications at a single location (seed yield) or two replications at each of two locations (other traits); single plot selection based on one plot at one location.
SE in parentheses.

proportion of nodules containing strains 61A76 and 123. These differences were not significant in 1992.

Error mean squares for seed yield for the three N treatments were homogeneous, in contrast to the random-line study, in which uninoculated plots had larger error terms. Although the main-effect differences among the three N treatments for seed yield were not significant (P=0.05), significant genotype x N treatment interactions occurred (Table 4). A few genotypes, such as Century 84, yielded less under the control treatment than in the treatments where N was provided through fertilizer or inoculation (Table 5). Other genotypes, such as Sandusky, showed no response to N. The two ways of providing N gave similar yields for most genotypes, but Conrad yielded more under inoculation than when fertilized (Table 5). Large interactions involving years and limited replication imply that the results for yield in this study should be interpreted with caution.

For protein content, results from the two years were different. In 1991, the mean protein content for the inoculated treatment was 422 g kg^{1} and that for the N fertilizer treatment was 421 g kg-^{1}, both differing significantly (P=0.05) from the mean of the control, 359 g kg-1. There were no significant differences among N treatments, in 1992 or in the two year combined analysis. Likewise genotype x N treatment interaction was non significant each year and in the combined analysis (Table 4). Genotypic differences for protein content were significant (P=0.05) each year and in the combined analysis (Table 4). Analysis of oil content revealed a pattern similar to that of protein, consistent with the negative correlation generally observed between these traits and reported by Helms *et al.*, 1998; Wilcox, 1998 and by Wilcox *et al.*, 2001. For 100-seed weight, maturity, plant height, and lodging, there were no significant effects of N treatments nor of no genotype x N treatment interactions.

observed on uninoculated plots in this study.Genotype x inoculation interactions appeared for protein content in the random-line test in 1991 and for seed yield in the cultivar test in the two-year analysis, but not for other traits. Expected genetic gains indicated that selection for protein would result in satisfactory progress irrespective of the type of selection or target environment. We conclude that genotype x inoculation interactions influence seed yield and composition in some environments but not others. Most cultivars responded identically to N whether it was supplied through symbiosis or fertilizer.

Table 3: Phenotypic correlations for 52 random lines from soybean population AP10C2, measured in the presence (right of diagonal) and absence (left of diagonal) of B. japonicum for two years (except 1 year for yield).

Trait	Maturity	Height	Lodging	Protein	Oil	Yield
Maturity		0.60**	0.52**	-0.03	-0.60**	0.11
Height	0.56**		0.69**	0.12	-0.19	0.30*
Lodging	0.41**	0.59**		-0.08	-0.37*	0.18
Protein	0.14	-0.08	-0.26		-0.25	0.17=
Oil	-0.71	-0.25	-0.25	-0.42**		0.15=
Yield	0.22	0.19	0.20	0.55*+=	-0.29*+=	

*,** significantly different from 0 at the 0.05 and 0.01 probability levels, respectively.
= significantly different at the 0.05 probability level from correlation between same traits in other inoculation treatment.

Table 4. Partial analysis of variance of seed yield and protein content for 12 soybean genotypes produced without added N (control), with B. japonicum (inoculated) and with N fertilizer

		Mean Square	
Source	df	Yield	Protein
N treatment	2	6.98	15 076
Control vs. N added	1	13.44	28 461
Inoculated vs. Fertilizer	1	0.51	1 692
N treatment x Year	2	2.50	17 827
Error a	4	0.57	3 009
Genotype	11	1.81	3 112**
Genotype x Year	11	0.65	409
Genotype x N treatment	22	0.56**	602
Control vs. N added	11	0.71**	21
Inoculated vs. Fertilizer	11	0.41*	482
Genotype x N treatment x Year	22	0.16	697
Error b	66	0.34	388

*,** significant at the 0.05 and 0.01 probability levels, respectively.

Table 5: Seed yield averaged across two years of 12 soybean genotypes produced without added N, inoculated with B. japonicum and with N fertilizer

			Seed Yield	
Genotype	No N	Inoculated	N Fertiliz	Mean
			t ha^{-1}	
Vinton 81	1.5=	2.3	2.7	2.2
Century 84	1.9	2.9	3.2	2.6
HS89-2958	2.3	2.9	3.0	2.7
HS89-2987	2.4	3.5	4.0	3.3
Resnik	2.9	3.4	3.4	3.3
Edison	2.6	3.1	3.5	3.1
Williams 82	3.2	3.6	3.8	3.5
Keller	2.4	2.5	2.5	2.5
Conrad	2.2	3.6	2.5	2.8
Kenwood	1.8	2.6	3.4	2.6
Sandusky	3.4	2.8	2.9	3.0
HS84-6247	2.6	2.9	3.0	2.8

= $LSD_{0.05}$ between N treatments for a given genotype = 0.9
$LSD_{0.05}$ between genotypes for a given N treatment =- 0.8
$LSD_{0.05}$ between different N treatments for different genotypes=

Table 5: Seed yield averaged across two years of 12 soybean genotypes produced without added N, inoculated with B. japonicum and with N fertilizer

Genotype	No N	Inoculated	Seed Yield N Fertiliz	Mean
			t ha^{-1}	
Vinton 81	1.5=	2.3	2.7	2.2
Century 84	1.9	2.9	3.2	2.6
HS89-2958	2.3	2.9	3.0	2.7
HS89-2987	2.4	3.5	4.0	3.3
Resnik	2.9	3.4	3.4	3.3
Edison	2.6	3.1	3.5	3.1
Williams 82	3.2	3.6	3.8	3.5
Keller	2.4	2.5	2.5	2.5
Conrad	2.2	3.6	2.5	2.8
Kenwood	1.8	2.6	3.4	2.6
Sandusky	3.4	2.8	2.9	3.0
HS84-6247	2.6	2.9	3.0	2.8

= $LSD_{0.05}$ between N treatments for a given genotype = 0.9

Conclusions

In both experiments there was a tendency for additional N, whether in fixed form or as fertilizer, to increase seed protein content but with little effect on seed yield. This indicates that, in the absence of *B. japonicum*, plants were able to derive substantial amounts of N directly from the soil. Yield increases of approximately one t ha^{-1} due to inoculation of seed were reported in Uganda (G. Gumisiriza and M. Mbalule, 1989, unpublished data). No such increases were observed in this experiment. It is possible that soybean growth on a weathered, tropical soil might be subjected to greater N stress than that

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